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Human Chorionic Gonadotropin-Induced Heterologous Desensitization of Adenylyl Cyclase from Highly Luteinized Rat Ovaries: Attenuation of Regulatory *N* Component Activity*

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ABSTRACT. We injected hCG into superovulated rats on the seventh day of pseudopregnancy and confirmed previous findings that this results in both homologous desensitization of luteal adenylyl cyclase (loss of responsiveness to LH) and heterologous desensitization of the same adenylyl cyclase system (partial loss of responsiveness to catecholamines), and that these changes are associated with the loss of available unoccupied LH receptors (down-regulation) but not with any discernible loss of β-adrenergic receptors.

We tested the hypothesis that the heterologous component of the above changes might be due to alterations in the function of the nucleotide-binding *N* component of adenylyl cyclase that intervenes between receptors and catalytic units of adenylyl cyclase. This was done by assessing *N* component activity in reconstitution assays that measured the capacity of luteal *N* to mediate, in *cyc*⁻ S49 lymphoma membranes, stimulation of adenylyl cyclase independently by the guanine nucleotide guanylyl imidodiphosphate, by NaF, or by the lymphoma membrane β-adrenergic receptor. By all of these modes of assay, heterologous desensitization of luteal adenylyl cyclase to β-adrenergic stimulation was found to be associated with a proportionally similar decrease in *N* component activity. This change in *N* component activity could be due to either quantitative or qualitative alterations. It is speculated that if the change is of a qualitative nature, the alteration may be a cAMP-dependent phosphorylation reaction of one of the subunits of the *N* component. (*Endocrinology* **113:** 1638, 1983)

CONTINUED occupancy of hormone receptor by hormone due to either continued presence of hormone or persistence of slowly dissociating hormone-receptor complex does not result in continued activation of the adenylyl cyclase system. Rather, as shown in response to gonadotropin stimulation of gonadal cells (1–7), a two-step process occurs whereby first the hormone-receptor complex becomes altered and uncouples from adenylyl cyclase, and then the receptor (alone or in combination with hormone) is removed from the cell surface (down-regulation). Hormone-induced desensitization of the adenylyl cyclase system occurs in two functionally and mechanistically different fashions. When the adenylyl cyclase loses responsiveness only to the hormone that induced the desensitization, the desensitization is termed homologous. When responsiveness is lost to other stimulating hormones as well, the desensitization is termed heterologous.

It has been reported that hCG-induced desensitization of luteal tissue in rats and rabbits is heterologous in nature (6–8). The injection of a single high dose of hCG into animals with functional corpora lutea (CL) not only causes a total loss of LH-stimulated adenylyl cyclase activity, but also results in partial but significant attenuation of adenylyl cyclase responses to NaF and the β-adrenergic agonist isoproterenol (Iso). Harwood *et al.* (7) reported that injection of superovulated rats with a bolus of hCG caused a drastic loss of LH receptor but no significant loss of β-adrenergic receptor. This latter result, together with their finding that epinephrine- and NaF-stimulated adenylyl cyclase activities were attenuated, led the authors to suggest that hCG-induced desensitization of β-adrenergic adenylyl cyclase responsiveness might be due to alterations in the component of the adenylyl cyclase system that couples hormone receptor to the catalytic unit of the system, *i.e.* the nucleotide-binding regulatory component, which we abbreviate as *N*.

As illustrated in the previous paper (9) as well as in other reports (11–14), it is possible to assess *N* component function by measuring its capacity to reconstitute adenylyl cyclase activity in membranes from the *cyc*⁻ variant of the S49 mouse lymphoma cell line which

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contain both membrane-bound β-adrenergic receptors and the catalytic unit of the adenylyl cyclase system, but are deficient in a functional stimulatory regulatory *N* component (10). Membranes from these cells can act, therefore, as recipients for *N* components from other tissue sources. In assays of this kind, *cyc*⁻ adenylyl cyclase activity is reconstituted to levels that are dependent upon the quantity and/or quality of the added *N* component. In the preceding article (9), we reported the validation of the assay for rabbit luteal *N* component. In the present report, we show that the same extraction- and assay procedures work equally well for *N* component from highly luteinized (ovarian) tissue from superovulated rats. Using this assay as well as specific binding assays for LH and β-adrenergic receptors, we explored the possible sites that were responsible for the altered responses of the ovarian adenylyl cyclase system after administration of a desensitizing dose of hCG.

Materials and Methods

Materials

All materials were the same as those described in the preceding article (9), with the addition of guanylyl imidodiphosphate [GMP-P(NH)P], which was purchased from Boehringer Mannheim (New York, NY).

Animals and treatment schedule

Rats were purchased from TIMCO (Houston, TX) and arrived at the animals facility at 26 days of age. All rats were injected with 50 IU PMSG at approximately 1000 h on day 30 of age. At about 1600 h on day 32 of age, all rats were injected with 50 IU hCG. One week later, on day 39 of age, 60 rats were injected with 75 IU hCG to initiate the desensitization process. Of these, 20 rats were killed about 8.5 h after the desensitizing hCG injection, 20 more were killed 27 h after injection, and the remaining 20 rats were killed 48 h after hCG injection. In addition, 20 rats that did not receive the desensitizating hCG injection were killed immediately after the other 60 rats were injected and served as the control group. This treatment schedule, which is illustrated in Fig. 1, was designed to provide data on the progression of the desensitization process over time. The rats were killed by cervical dislocation, and the ovaries were removed and placed in ice-cold Krebs-Ringer bicarbonate buffer prepared with half the recommended amount of $CaCl_2$ (15). The ovaries of rats from each of the 4 groups were pooled, and homogenization and membrane particle preparation were performed, as described previously (16).

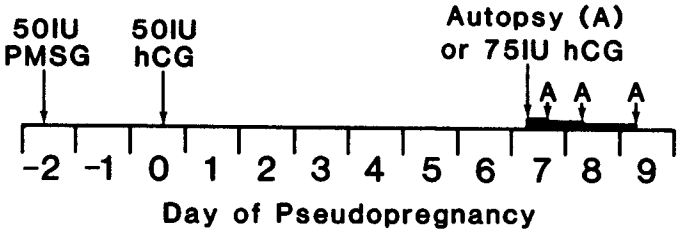

FIG. 1. Treatment schedule for the study of hCG-induced desensitization in superovulated rat ovarian tissue. Day −2 of pseudopregnancy corresponds to day 30 of age. Rats were killed (A) 18.5, 27, and 48 h after the 75-IU hCG injection.

Methods

All assays, the preparation of *cyc*⁻ membranes, and the extraction of ovarian *N* component were as described in the preceding article (9), with the following exceptions: 1) GMP-P(NH)P (60 μM in the assay) was used (in addition to the substances already described) to stimulate adenylyl cyclase in both ovarian membranes and the reconstituted *cyc*⁻ system, and 2) heat inactivation of cholate extract catalytic unit was carried out at 32.5 C for 5 min and at room temperature for 25 min.

Results

Initial study

To test the hypothesis of Harwood *et al.* (7) that hCG-induced heterologous desensitization of highly luteinized ovarian tissue involves an alteration of *N* component function, an initial study was performed using the procedures described in *Materials and Methods*, except the rats were killed 18 h after hCG injection instead of at 8.5, 27, and 48 h, and the $MgCl_2$ concentration in the assay for *N* component activity was 1.4 mM instead of 20 mM. LH and Iso were added to the adenylyl cyclase assays to assess homologous and heterologous effects, respectively, of the desensitizing hCG injection on hormone-responsive adenylyl cyclase activity. NaF was added to the assays to determine the effects of desensitization on hormone receptor-independent activation of adenylyl cyclase. The results of this study are given in Table 1. The data verify the previously reported findings (6–8) that hCG injection causes not only a suppression of LH-stimulated adenylyl cyclase activity, but also causes a decrease in available LH receptors, with no change in β-adrenergic receptors, and attenuates Iso- and NaF-stimulated adenylyl cyclase activity as well. In addition, the data suggest that, indeed, hCG treatment causes alteration of *N* component function, as indicated by a decreased level of reconstituted *cyc*⁻ adenylyl cyclase activity in response to Iso binding to the *cyc*⁻ β-adrenergic receptor. The values reported for the reconstitution assay in Table 1 are lower than those reported below because of the low Mg ion concentration. The data reported below are from more extensive studies of this phenomenon.

Adenylyl cyclase activities and binding data

To verify further that hCG treatment was effective in producing a heterologous desensitization over a prolonged period of time, more extensive adenylyl cyclase

TABLE 1. Effects of hCG injection on adenylyl cyclase activity, hormone binding, and *N* component activity in membranes of superovulated rat ovaries

Assay	Treatment of rats	
	None	18 h after hCG
A. Adenylyl cyclase activities (pmol/min·mg)		
Control (95 μM GTP)	12 ± 1	16 ± 1
LH + GTP	145 ± 5	39 ± 1
Iso + GTP	132 ± 1	102 ± 5
B. Specific receptor binding (fmol/mg)		
[^{125}I]Iodo-hCG (5.0 nM)	660 ± 12	15 ± 1
[^{125}I]IHYP (5.0 nM)	78 ± 2	76 ± 7
C. *N* Activity (pmol cAMP formed/mg extract protein in 10 min by *cyc*$^-$ membranes in the presence of Iso + GTP	22 ± 1	17 ± 1

Superovulated rats received 75 IU hCG, ip, on the afternoon of the seventh day of psuedopregnancy and were killed 18 h afterward. Control rats (no treatment) were killed on the afternoon of the seventh day of pseudopregnancy. Ovary removal, membrane preparation, adenylyl cyclase assays, specific binding assays of [^{125}I]iodo-hCG to LH/hCG receptors and of [^{125}I]IHYP to β-adrenergic receptors, and extraction and assay of *N* activity by reconstitution of Iso-stimulated adenylyl cyclase activity in *cyc*$^-$ membranes were performed as described in *Materials and Methods*. Assays were performed on membranes prepared from the pools of ovaries of five rats per group. Values are the means ± SEM of triplicate determinations of each pool.

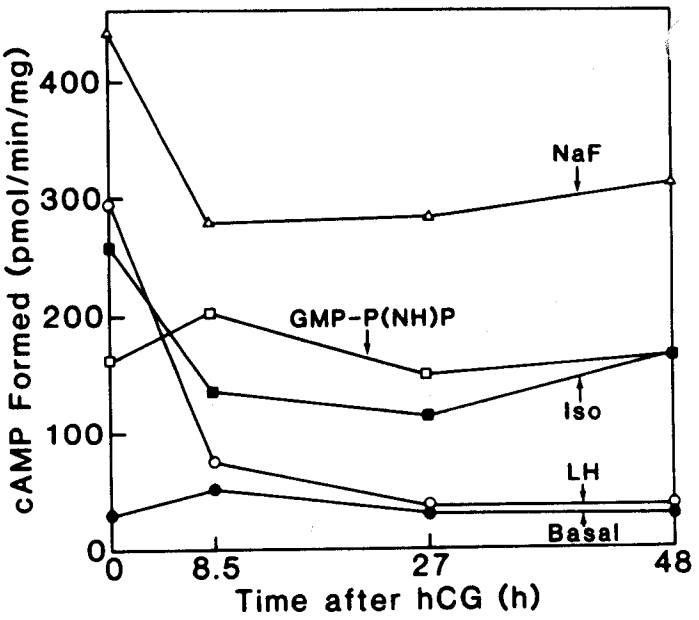

FIG. 2. Response of the highly luteinized ovarian adenylyl cyclase system to LH, Iso, GMP-P(NH)P, and NaF before and after a desensitizing injection of hCG. When present, the concentration of LH was 10 μg/ml, that of Iso was 100 μM, that of GMP-P(NH)P was 60 μM, and that of NaF was 10 mM. Tubes used to measure basal, LH, and Iso activities also contained 95 μM GTP.

TABLE 2. Effect of hCG injection of superovulated rats on adenylyl cyclase activities in ovarian membranes

Time after hCG	Adenylyl cyclase activities in the presence of			
	GTP	GTP + LH	GMP-P(NH)P	GMP-P(NH)P + LH
None	25 ± 2	313 ± 16	187 ± 4	668 ± 26
8.5 h	45 ± 3	73 ± 2	217 ± 5	262 ± 4
27 h	28 ± 1	40 ± 1	157 ± 5	186 ± 2

Rats did (hCG-treated) or did not (control) receive 50 IU hCG on the morning of the seventh day of pseudopregnancy and were killed either then (control) of 8.5 or 27 h afterward (hCG-treated). The ovaries from the rats of each group were pooled, and membranes were prepared from the ovarian pools and assayed for adenylyl cyclase activities in the presence of the additives shown under the conditions described in *Materials and Methods*.. When present, the concentration of LH was 10 μg/ml, and that of both GTP and GMP-P(NH)P was 95 μM. Values are the means ± SEM of triplicate determinations of each pool, expressed as picomoles per min/mg.

and receptor binding assays were performed. The data from the adenylyl cyclase assays are presented in Fig. 2 and Table 2. By 8.5 h after the desensitizing hCG injection, LH-stimulated adenylyl cyclase activity had decreased by approximately 75% relative to the control value (0 h) and was barely above the basal level 27 and 48 h after treatment (Fig. 1). This effect of hCG treatment on LH-stimulated activity is observable not only when assays are carried out in the presence of GTP (Fig. 1) but also when the nonhydrolyzable analog GMP-P(NH)P is used as the added nucleotide (Table 2). The response of the adenylyl cyclase system to Iso assayed in the presence of either GTP (Fig. 1) or GMP-P(NH)P (not shown) was also decreased relative to the control value but to a much lesser extent. The decreases in the presence of GTP in the Iso response were 45%, 55%, and 35% relative to control values 8.5, 27, and 48 h after hCG treatment, respectively. NaF responsiveness followed a pattern similar to that of Iso. In contrast to the effects noted above, both basal (GTP) and GMP-P(NH)P-stimulated activities were somewhat elevated at the 8.5 h time point (Fig. 1 and Table 2), but were at control levels 27 and 48 h after hCG treatment.

We tested whether the effects of the hCG treatment were associated with alterations in the concentrations required for LH or Iso to elicit half-maximal stimulations of adenylyl cyclase activity (EC_{50}) or with changes in ovarian LH or β-adrenergic receptor levels. As illustrated in Fig. 3, the hCG-induced decreases in hormonal responses observed in the experiment shown in Fig. 1 were not due to shifts in the EC_{50} value at which either LH (*top panel*) or isoproterenol (*bottom panel*) stimulated the ovarian adenylyl cyclase system's response to hormones. Upon testing the levels of LH and β-adrenergic receptors in these membranes, we found that while hCG treatment resulted in a marked (~60% at 8.5 h and more than 90%

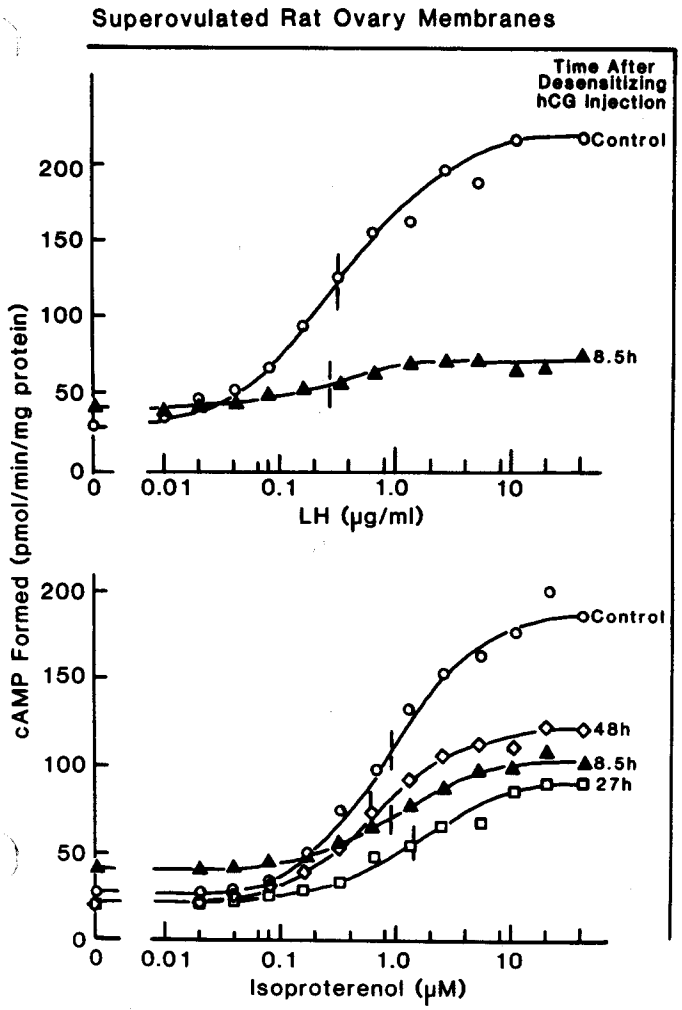

FIG. 3. LH (*top panel*) and Iso (*bottom panel*) dose-response analyses of adenylyl cyclase activity in highly luteinized ovarian membranes of control and hCG-desensitized rats. The *vertical lines bisecting each curve* are at the EC_{50} points. Curves for the 27 and 48 h post-hCG membranes are not included in the upper graph because, in essence, there is no response to LH above the basal level in either group. GTP was present in all assay tubes at a concentration of 95 μM.

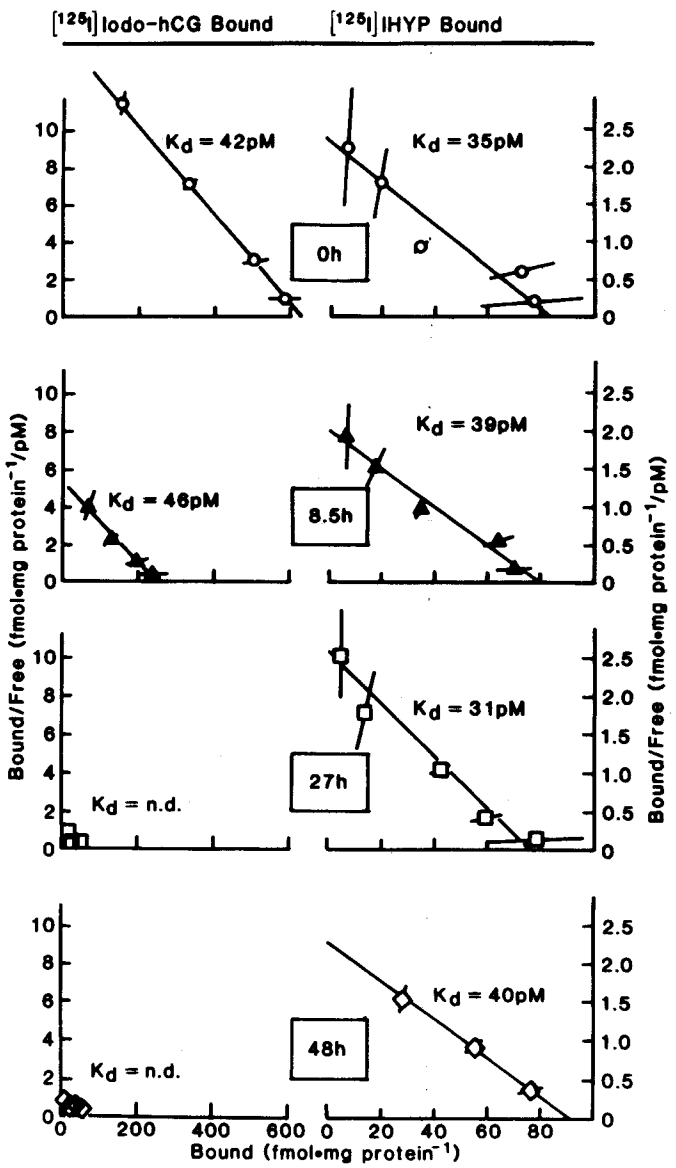

FIG. 4. Scatchard plots of [125I]iodo-hCG (*left four plots*) and [125I] IHYP (*right four plots*) binding to membranes prepared from highly luteinized ovaries of control (0 h) and hCG-desensitized superovulated rats. The x-intercept of each plot represents the B_{max}. The *error bars through each point* are the SEM of triplicate determinations. n.d., The K_d and B_{max} could not be determined accurately due to the apparent lack of available receptors.

at 27 and 48 h) decrease in available LH/hCG receptors (Fig. 4, *left panel*, and Fig. 5), the level of β-adrenergic receptors appeared to drop only slightly when measured at 8.5 and 27 h (5–10% decrease relative to the control value) and gradually increased to slightly elevated (10%) levels relative to the control value by 48 h (Fig. 4, *right panel*, and Fig. 5), with none of these changes being statistically significant. Binding affinities did not appear to be altered to any great extent by the hCG treatment.

In an attempt to clarify the contrasting results obtained for GMP-P(NH)P-stimulated adenylyl cyclase activity and NaF-stimulated adenylyl cyclase activity (Fig. 2), we studied the characteristics of the GMP-P(NH)P response further by testing the effects of Mg concentration on the rate of activation of adenylyl cyclase activity. The results are shown in Fig. 6. In agreement with previously reported results (17, 18), stimulation of luteal adenylyl cyclase by GMP-P(NH)P occurred with a lag, and Mg ion decreased this lag in a concentration-dependent manner (Fig. 6, A *vs.* B *vs.* C) regardless of whether it acted on membranes from control or hCG-treated rats. The two membrane preparations differed, however, in that 1) at the higher concentrations of Mg tested, the steady state GMP-P(NH)P-stimulated activities were higher in the membranes from hCG-treated rats than in membranes from control rats, and 2) at the

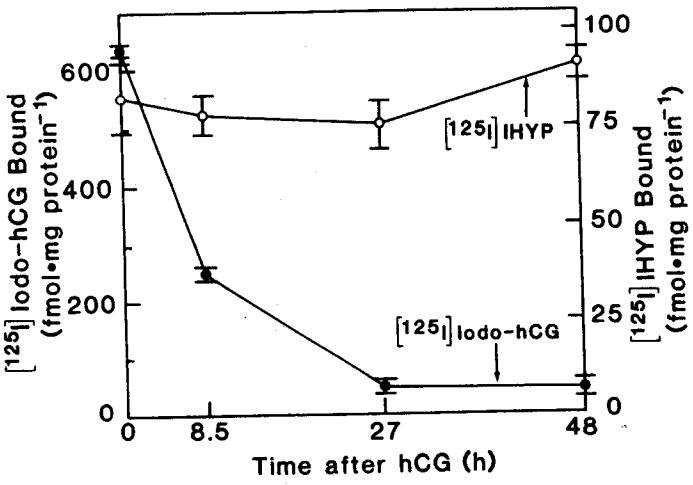

FIG. 5. Maximum binding of [^{125}I]iodo-hCG and [^{125}I]IHYP to highly luteinized ovarian membranes prepared from control and hCG-desensitized superovulated rats. The B_{max} values were derived from Scatchard analysis, and the *error bars* represent the SEs, of the estimates of B_{max} based on simple linear regression analysis.

low Mg concentration (0.3 mM in excess of ATP plus EDTA), but not at high concentrations (3.8 and 18.8 mM in excess of ATP plus EDTA), GMP-P(NH)P-stimulated adenylyl cyclase activity reached steady state faster in the membranes from hCG-treated rats than in those from control rats.

N component activity

Since it was apparent that the Iso-responsive adenylyl cyclase system was uncoupled due to hCG treatment (decreased adenylyl cyclase activity despite control levels of β-adrenergic receptors), an investigation of *N* component function in the membranes from control and hCG-desensitized rats was initiated. Figure 7 shows the cyc^- reconstituting activities present in cholate extracts prepared from control and hCG-treated (27-h) highly luteinized rat ovarian membrane pools. Reconstituted cyc^- adenylyl cyclase activity is plotted against micrograms of luteal cholate extract protein added to the assay. All assays were linear over the range of cholate extract protein used. hCG treatment caused an approximate 35% decrease in reconstituted adenylyl cyclase activity relative to the control value regardless of whether reconstitution of stimulation of cyc^- activity by Iso plus GTP, NaF, or GMP-P(NH)P (Fig. 7, A, B, and C, respectively) was used to assess *N* component activity.

Figure 8 shows the complete time course of variation in *N* component activity in ovarian tissue after hCG injection. The changes in *N* component activity were similar regardless of mode of assay, with hCG injection causing decreases of about 20% relative to the control value of 8.5 h and approximately 35–40% relative to the control value at 27 and 48 h.

Discussion

Hormone-induced desensitization can occur in one of two fashions. The first is a rapid homologous desensitization which involves an uncoupling of hormone-receptor complex from the *N* component of the adenylyl

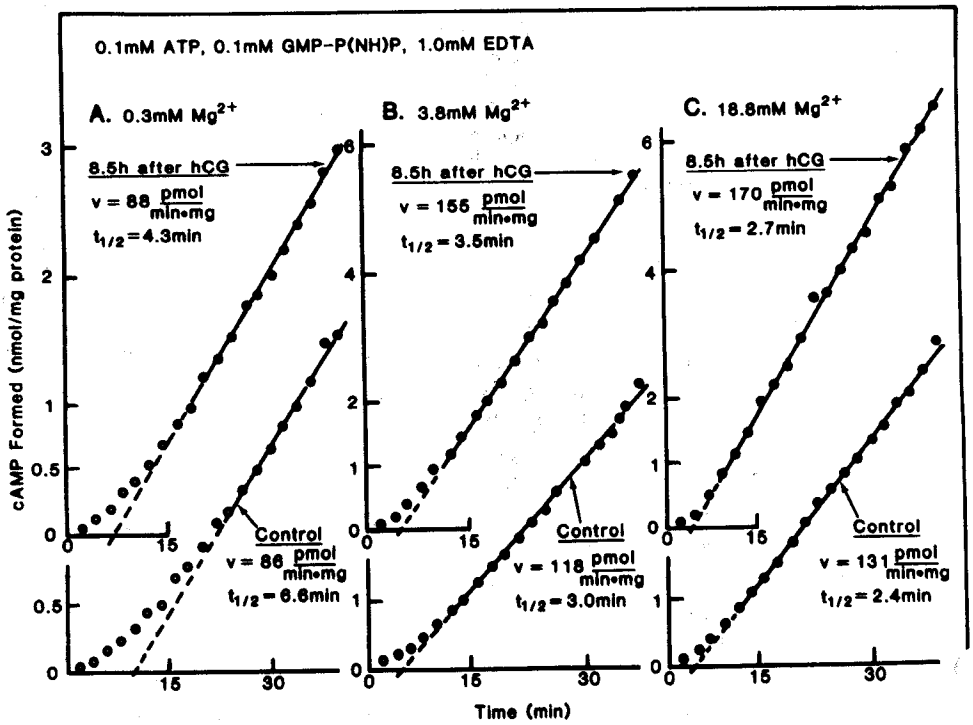

FIG. 6. Effects of varying Mg^{++} ion concentration on the time course of activation of the adenylyl cyclase system to GMP-P(NH)P in membranes prepared from highly luteinized ovaries of control and hCG-desensitized rats (8.5 h). The steady state velocity (v) of cAMP formation in membranes from control rats was about the same (A) or less than (B and C) that in membranes from hCG-densitized rats. The $t_{½}$ of the lag time to steady state activity in membranes from control rats was about the same (B and C) or greater (A) than that in membranes from hCG-desensitized rats. The net result was always a greater amount of cAMP formed per min/mg protein in membranes from hCG-desensitized rats than in membranes from control rats.

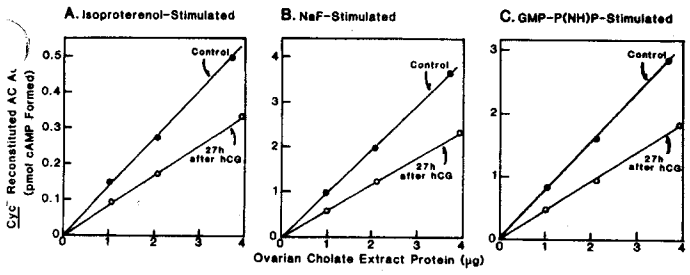

FIG. 7. Linearity of reconstitution assays with respect to cAMP formed by the *cyc*⁻ adenylyl cyclase system reconstituted with varying amounts of *N* component-containing cholate extract protein. Linearity exists over the range of cholate extract protein used (1–4 μg) whether the source of the extract is from control or hCG-desensitized rat ovaries and whether the reconstituted system is stimulated with Iso (A), NaF (B), or GMP-P(NH)P (C).

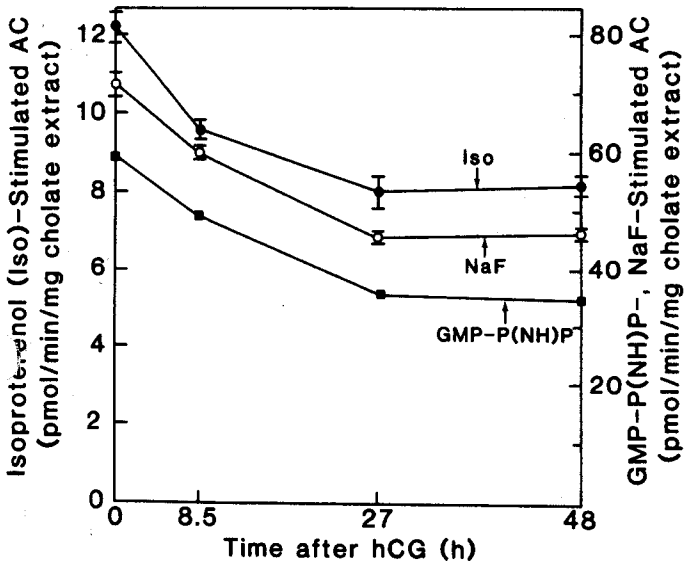

FIG. 8. Time course of the effect of hCG desensitization on Iso-, NaF-, and GMP-P(NH)P-stimulated highly luteinized ovarian *N* component function. *N* Component function was assessed by reconstitution of *cyc*⁻ adenylyl cyclase activity with *N* component-containing cholate extracts of control and hCG-desensitized highly luteinized ovarian membranes. Each *point* represents the mean ± SEM of results from three separate assays.

cyclase system (1–7) with no apparent alteration of *N* component function (19). This type of desensitization can be mimicked *in vitro* requiring Mg and ATP (20, 21), adenylyl imidodiphosphate cannot be substituted for ATP (20, 21), and can be reversed with a phosphoprotein phosphatase (22). All of these facts taken together suggest that a phosphorylation may be involved in homologous desensitization. Since there is no alteration in *N* component function upon homologous desensitization (19) and since this type of desensitization is hormone specific, it has been proposed that homologous desensitization is due to an alteration in the receptor, possibly phosphorylation, which causes the uncoupling from the rest of the adenylyl cyclase system. It is also possible that the proposed phosphorylation targets the receptor for the subsequent internalization that occurs. It has been demonstrated that actual receptor loss in rat testicular tissue does not occur until 24–48 h after hCG injection and is not maximal until about 72 h after injection (4). In the ewe, LH injection does not cause the loss of luteal LH/hCG receptor until 12–24 h after injection (23). Uncoupling, however, takes place within hours, as assessed by homologous loss of LH-stimulated adenylyl cyclase activity in rabbit luteal tissue (2) and follicular tissue (3).

The second type of desensitization is heterologous in nature and probably includes all of the events that occur in homologous desensitization and, in addition, a much slower loss of response to other hormones and NaF. Whereas the rapid homologous desensitization may be cAMP independent (20), the slower heterologous desensitization may be cAMP dependent (24–26). The fact that there is a loss of responsiveness to NaF, a substance known to elicit adenylyl cyclase activity by a direct effect on the *N* component (11, 27), prompted us to investigate *N* component function in hCG-induced heterologous desensitization.

The ability of the regulatory *N* component of the adenylyl cyclase system to activate the catalytic unit is dependent upon the presence of Mg (10, 17, 28) and guanine nucleotide (10, 29, 30). The K_m of the *N* component for GTP is in the micromolar range, which is substantially below the intracellular concentration of GTP (31, 32). However, even though the *N* component is saturated by guanine nucleotide, its activation is slow, with a lag time regulated by the Mg ion concentration. This is because under basal conditions in the absence of hormone, the K_m of the *N* component for Mg is about 5 mM, which is well above the approximate 0.5-mM intracellular concentration of Mg (10, 17). As a result, in the absence of hormone, the *N* component is, for the most part, in an inactive form. In the presence of hormone, hormone-receptor complex interaction with the *N* component serves both to lower its K_m for Mg to a value of about 10 μM (28), allowing the *N* component to assume its active form much more readily, and to enhance the extent to which the *N* component can be activated at saturating concentrations of Mg ion, leading to higher maximal *N* component activities than could be elicited by the mere addition of excess Mg ion (28).

As can be seen in Fig. 2, 8.5 h after hCG injection, the basal (GTP-stimulated) activity is somewhat elevated with respect to the control value, suggesting that there is more *N* component in the active form. Similarly, the GMP-P(NH)P-stimulated activity is also elevated at 8.5 h with respect to the control value. However, extracted *N* component from the 8.5 h as well as other time points exhibited decreased activity when assayed in *cyc*⁻ mem-

branes regardless of the stimulatory ligand used [*i.e.* GMP-P(NH)P, NaF, or occupied hormone receptor plus GTP].

One explanation for the slightly increased (nucleotide-stimulated) activities measured in intact membranes after desensitization is that, in fact, these activities are the result of the combined actions of nucleotides plus residual hormonal stimulation due to long-lived hCG, which, due to tight binding (33), would not have washed off membranes during their preparation and could still elicit partial effects. The experiment shown in Fig. 6 provided data consistent with this thought. This experiment showed 1) that when ovarian membranes from rats that had received hCG 8.5 h before death were tested for their response to saturating GMP-P(NH)P under limiting Mg ion concentrations (Fig. 6A), they showed a short lag in responding to the nucleotide, as expected from an adenylyl cyclase system with a response to Mg that is facilitated by the presence of hormone-receptor complex (28); and 2) that upon adding sufficient Mg ion to the assay so as to obliterate the effect of the pretreatment on the lag in the responsiveness of the system to GMP-P(NH)P (Fig. 6, B and C), the steady state activities achieved after full stimulation by the nucleotide were indeed increased in the membranes of ovaries from hCG-treated rats compared to that in the control group.

The ineffectiveness of LH to stimulate adenylyl cyclase activity in hCG-desensitized rat ovaries in animals injected with hCG 8.5 h previously can, therefore, be explained by a combined effect of uncoupling of receptor from the regulatory component of the adenylyl cyclase system, which probably results from an alteration (phosphorylation) of receptor, and a decrease in *N* component activity. After longer periods of desensitization (27 and 48 h), receptor alteration and down-regulation of LH/hCG receptors can almost totally explain the overall effect seen. Thus, other than the comparatively minor contribution of decreased *N* component activity, the hCG-induced loss of LH responsiveness has the characteristics of homologous desensitization due to receptor alteration. On the other hand, there are no significant changes in β-adrenergic receptors. Yet, adenylyl cyclase activity is attenuated in membranes from hCG-treated rats to an extent similar to that to which *N* activity assayed by three different reconstitution assays is decreased. This indicates that this heterologous type of desensitization is best explained as being due to coupling protein alteration with no discernible effects on receptor function, which implies that there is no distinction between the *N* component that associates with LH receptor and the *N* component that associates with β-adrenergic receptor. This is in agreement with the assessment of Abramowitz and Birnbaumer (18) that LH and Iso act via the same adenylyl cyclase system distal to hormone receptor in luteal tissue. Figure 9 summarizes our findings that hCG treatment resulted in no changes in β adrenergic receptor while causing partial losses in both Iso-stimulable luteal adenylyl cyclase and regulatory *N* component activities.

The finding that *N* component activity is affected by heterologous desensitization in a manner that is not distinguishable in terms of its mediation of nucleotide, NaF, or hormone-receptor stimulation in *cyc*⁻ membranes is of interest. It indicates that the alteration in its function is not related to the sites of interaction of either hormone receptor or nucleotide, but, rather, to the inherent activation process. The results illustrated in Fig. 6 show further that of the two key parameters known to regulate *N* activity, *i.e.* Mg regulation of rate of activation and extent to which *N* can be activated, Mg regulation of the rate still appears to be operative, with essentially identical rates of activation being achieved upon the addition of excess Mg ion in the membranes from hCG-treated and control rat ovaries. Thus, it appears that the alteration in *N* component activity leading to heterologous desensitization lies in the extent to which the regulatory protein can be activated to stimulate the catalytic unit of the adenylyl cyclase system. However, the results could also be explained if there were a quantitative loss of *N* component, possibly due to internalization or total inactivation of a fixed proportion of *N* o

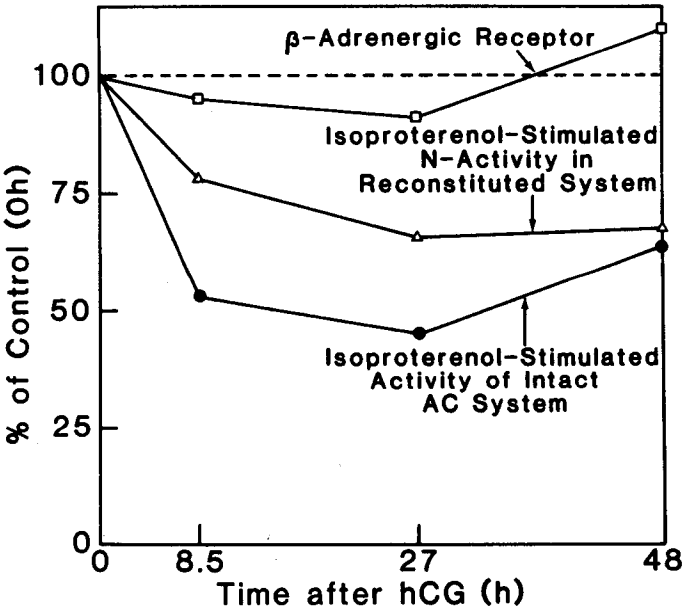

FIG. 9. Summary of the time course of effects of hCG-induced desensitization of the β-adrenergic responsive adenylyl cyclase system in membranes from highly luteinized ovaries. Data are presented for β-adrenergic binding sites as a percentage of the control (0 h) reconstitution of *cyc*⁻ Iso-stimulated adenylyl cyclase activity with ovarian *N* component (a measure of *N* component function) and Iso-stimulated ovarian membrane adenylyl cyclase activity (a measure of responsiveness of the intact adenylyl cyclase system to Iso) and is plotted.

one of its subunits. The recent report by Kassis and Fishman (14) sheds some light on this question. These investigators found that *N* component activity was reduced in prostaglandin E_1-desensitized human fibroblasts when assessed by reconstitution of cyc^- adenylyl cyclase as we have done, but that cholera toxin substrate levels, assessed by ADP-ribosylation with [^{32}P]NAD$^+$, which measures the α-subunit of stimulatory *N*, were unaltered. Taken together with our findings, these data would suggest that heterologous desensitization is the result either of a qualitative change in *N* or a quantitative change in the non-ADP-ribosylated β-subunit of *N*. In view of the findings that treatment of a variety of cells (24, 25), including rabbit Graafian follicular cells (26), with cAMP or cAMP-enhancing drugs, such as cholera toxin, may result in a nonspecific (heterologous) desensitization of the adenylyl cyclase system to hormonal stimulation, we currently favor the interpretation that heterologous desensitization is the result of a qualitative change in *N*, possibly its capacity to dissociate into its subunits in response to saturating nucleotides and Mg, which would be expected to lead to the decrease in extent of activation found experimentally. This alteration may be a phosphorylation of one of the *N* subunits. However, further studies are necessary to prove this hypothesis.

In summary, luteinized ovarian tissue of superovulated rats injected with a bolus of hCG desensitize in a heterologous manner. The near total loss of LH responsiveness can be explained by a near total loss of available LH/hCG receptor sites. β-Adrenergic receptor content does not change appreciably and, therefore, cannot account for the attenuated response of the adenylyl cyclase system to Iso. However, the decrease in ovarian *N* component activity described above can account for the loss of Iso responsiveness. It is our belief that the decrease in *N* activity is the result of a qualitative alteration in *N*, possibly a cAMP-dependent phosphorylation. We are currently investigating this possibility.

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References

1. Marsh JM, Mills TM, LeMaire WJ 1972 Cyclic AMP synthesis in the rabbit Graafian follicle and the effect of luteinizing hormone. Biochim Biophys Acta 273:389
2. Hunzicker-Dunn M, Birnbaumer L 1976 Adenylyl cyclase activities in ovarian tissues. IV. Gonadotrophin-induced desensitization of the luteal adenylyl cyclase throughout pregnancy and pseudopregnancy in the rabbit and the rat. Endocrinology 99:211
3. Hunzicker-Dunn M 1981 Rabbit follicular adenylyl cyclase activity. II. Gonadotropin-induced desensitization in granulosa cells and follicle shells. Biol Reprod 24:279
4. Hseuh AJW, Dufau ML, Catt KJ 1977 Gonadotropin-induced regulation of luteinizing hormone receptors and desensitization of testicular 3′5′-cyclic AMP and testerone responses. Proc Natl Acad Sci USA 74:592
5. Lamprecht SA, Zor U, Salomon Y, Koch Y, Ahren K, Linder HR 1977 Mechanism of hormonally induced refractoriness of ovarian adenylate cyclase to luteinizing hormone and prostaglandin E_2. J Cyclic Nucleotide Res 3:69
6. Hunzicker-Dunn M, Day SL, Abramowitz J, Birnbaumer L 1979 Ovarian responses of pregnant mare serum gonadotropin- and human chorionic gonadotropin-primed rats: desensitization, luteolytic, and ovulatory effects of a single dose of human chorionic gonadotropin. Endocrinology 105:442
7. Harwood JP, Richert ND, Dufau ML, Catt KJ 1980 Gonadotropin-induced desensitization of epinephrine action in the luteinized rat ovary. Endocrinology 107:280
8. Kirchick HJ, Birnbaumer L 1981 Prostaglandins do not appear to play a role in hCG-induced regression or desensitization of rabbit corpora lutea. Biol Reprod 24:1006
9. Kirchick HJ, Birnbaumer L 1983 Effects of estradiol treatment on rabbit luteal adenylyl cyclase: loss of luteinizing hormone receptors and attenuation of the regulatory *N* component activity. Endocrinology 113:1629
10. Bourne HR, Coffino P, Tomkins GM 1975 Selection of a variant lymphoma cell deficient in adenylate cyclase. Science 187:750
11. Iyengar R 1981 Hysteretic activation of adenylyl cyclases. II. Mg ion regulation of the activation of the regulatory component as analyzed by reconstitution. J Biol Chem 256:11042
12. Ross EM, Howlett AC, Ferguson KM, Gilman AG 1978 Reconstitution of hormone-sensitive adenylate cyclase activity with resolved components of the enzyme. J Biol Chem 253:6401
13. Farfel Z, Brickman AS, Kaslow HR, Brothers VM, Bourne HR 1980 Defect of receptor-cyclase coupling protein in pseudohypoparathyroidism. N Engl J Med 303:237
14. Kassis S, Fishman PH 1982 Different mechanisms of desensitization of adenylate cyclase by isoproterenol and prostaglandin E_1 in human fibroblasts. J Biol Chem 257:5312
15. Cohen RP 1957 Suspending media for animal tissues. In: Umbriett WW, Burris RH, Stauffer JF (eds) Manometric Techniques. Burges, Minneapolis, p 149
16. Birnbaumer L, Yang P-Ch, Hunzicker-Dunn M, Bockaert J, Duran JM 1976 Adenylyl cyclase activities in ovarian tissues. I. Homogenization and conditions of assay in Graafian follicles and corpora lutea of rabbits, rats and pigs: regulation by ATP, and some comparative properties. Endocrinology 99:163
17. Iyengar R, Birnbaumer L 1981 Hysteretic activation of adenylyl cyclases. I. Effect of Mg ion on the rate of activation by guanine nucleotides and fluoride. J Biol Chem 256:11036
18. Abramowitz J, Birnbaumer L 1982 Properties of the hormonally responsive rabbit luteal adenylyl cyclase: effects of guanine nucleotides and magnesium ion on stimulation by gonadotropin and catecholamines. Endocrinology 110:773
19. Iyengar R, Bhat MK, Riser ME, Birnbaumer L 1981 Receptor-specific desensitization of the S49 lymphoma cell adenylyl cyclase. Unaltered behavior of the regulatory component. J Biol Chem 256:4810
20. Bockaert J, Hunzicker-Dunn M, Birnbaumer L 1976 Hormone-stimulated desensitization of hormone-dependent adenylyl cyclase. Dual action of luteinizing hormone on pig Graafian follicle membranes. J Biol Chem 251:2653
21. Iyengar R, Mintz PW, Swartz TL, Birnbaumer L 1980 Divalent cation-induced desensitization of glucagon stimulable adenylyl cyclase in rat liver plasma membranes: GTP-dependent stimulation by glucagon. J Biol Chem 255:11875
22. Hunzicker-Dunn M, Derda D, Jungmann RA, Birnbaumer L 1979 Resensitization of the desensitized follicular adenylyl cyclase system to luteinizing hormone. Endocrinology 104:1785
23. Suter DE, Fletcher PW, Sluss PM, Reichert Jr LE, Niswender GD 1980 Alterations in the number of ovine luteal receptors for LH and progesterone secretion induced by homologous hormone. Biol Reprod 22:205
24. Su YF, Cubeddu X, Perkins JP 1976 Regulation of adenosine 3′:5′-monophosphate content in human astrocytoma cells: desensitization to catecholamines and prostaglandins. J Cyclic Nucleotide Res 2:257

25. Nichols GA, Brooker G 1979 Induction of refractoriness to isoproterenol by prior treatment of C6-2B rat astrocytoma cells with cholera toxin. J Cyclic Nucleotide Res 5:435
26. Hunzicker-Dunn M, Birnbaumer L 1981 Studies on the mechanism of luteinizing hormone-induced desensitization of the rabbit follicular adenylyl cyclase system *in vitro*. Endocrinology 109:345
27. Ross EM, Gilman AG 1977 Resolution of some components of adenylate cyclase necessary for catalytic activity. J Biol Chem 252:6966
28. Iyengar R, Birnbaumer L 1982 Hormone receptor modulates the regulatory component of adenylyl cyclase by reducing its requirement for Mg^{2+} and enhancing its extent of activation. Proc Natl Acad Sci USA 79:5179
29. Pfeuffer T 1977 GTP-binding proteins in membranes and the control of adenylate cyclase activity. J Biol Chem 252:7224
30. Sternweis PC, Northup JK, Smigel MD, Gilman AG 1981 The regulatory component of adenylate cyclase. Purification and properties. J Biol Chem 256:11517
31. Iyengar R, Birnbaumer L 1979 GDP promotes coupling and activation of cyclizing activity in the glucagon-sensitive adenylate cyclase system of rat liver plasma membranes. Evidence for two levels of regulation in adenylyl cyclase. Proc Natl Acad Sci USA 76:3189
32. Iyengar R, Abramowitz J, Bordelon-Riser ME, Blume AJ, Birnbaumer L 1980 Regulation of hormone-receptor coupling to adenylyl cyclase: effects of GTP and GDP. J Biol Chem 255:10312
33. Abramowitz J, Birnbaumer L 1982 Temporal characteristics of gonadotropin interaction with rabbit luteal receptors and activation of adenylyl cyclase: comparison to the mode of action of catecholamine receptors. Endocrinology 111:970